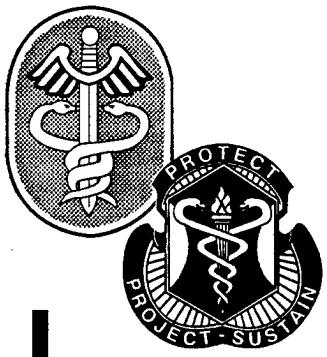

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Serial Probe Recognition as an Environmental Enrichment Device for Nonhuman Primates

Tracy L. Copeland

June 1997

U.S. Army Medical Research
Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010-5425

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Commander
U.S. Army Medical Research Institute of Chemical Defense
ATTN: MCMR-UV-PA
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5425

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In the present study, a Serial Probe Recognition (SPR) task was evaluated as an environmental enrichment device for nonhuman primates. Twenty-one rhesus monkeys were assigned to two experimental groups, one group male (n=15) and one group female (n=6). Urinary cortisol levels, which correlate postively with stress, were measured to determine the effectiveness of SPR as an environmental enrichment device, with baseline measurements taken before the onset of the task, and experimental measurements taken during SPR training. Results showed that cortisol levels were significantly higher during the SPR condition than during the baseline condition. There was also a significant difference between 1st collection and 2nd collection (labelled 1st and 2nd) cortisol levels during the SPR condition, with 2nd cortisol levels being higher than 1st cortisol levels. In addition, there was a significant interaction of treatment condition and time of day during the SPR condition, in that 2nd cortisol levels during the SPR condition were higher than the 2nd cortisol levels during the baseline condition. A series of planned contrasts were executed and described. The best models to explain why the animals experienced an increase in cortisol levels are the assertions that 1) the animals had not habituated to the SPR task when experimental measurements were taken and 2) the animals experienced increased arousal necessary to perform a novel task. Both of these premises would explain the increases in stress levels and the increases in cortisol levels.

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Introduction

The Animal Welfare Act Amendment (1985) was established to lessen the effects of psychological stress that sustained captivity would engender on the research animal. This amendment requires researchers to provide "a physical environment adequate to promote psychological well-being of primates" (Animal Welfare Act, 1985, p. 1752). In addition to the Animal Welfare Act (1985), the regulations published by the Animal and Plant Health Inspection of the U.S. Department of Agriculture (USDA) contain portions devoted to environmental enrichment for the promotion of the psychological well-being of nonhuman primates. The regulations require that "dealers, exhibitors, and research facilities must develop, document, and follow an appropriate plan for environmental enhancement," (Federal Register, 1991, p. 6471), and the plan must include methods for the animals' social grouping, environmental enrichment, and the utilization of restraint devices. Furthermore, special considerations must be made for young, activity restricted, or distressed animals, as well as individually housed nonhuman primates without visual or auditory access to others of their species.

There are many ways in which laboratory environments can be enriched to promote nonhuman primates' well being. These features can be classified as physical, nonsocial, and social aspects of the laboratory environment. In terms of the physical environment, there are regulations to which laboratories must adhere, such as light intensity, temperature/humidity range, air exchange, and cage size (Animal Welfare Act, 1985). Another physical factor to consider is the availability of foraging opportunities (Novak, Rulf, Munroe, Parks, Price, O'Neill, & Suomi, 1995). A way to observe the effect of the physical variables of nonhuman primates' environment is to determine the influence on their physical health (Keeling & Wolfe, 1975; Kerr, Scheffler, & Waisman, 1969; Novak & Suomi, 1988). As for nonsocial aspects, manipulanda (Ross & Everitt, 1988; Schapiro, Brent, Bloomsmith, & Satterfield, 1991) and electronic or computerized devices can also enrich the environment for nonhuman primates (Markowitz, 1979; Rumbaugh, 1989; Schrier, Angarella, and Povar, 1984). Lastly, nonhuman primates have been shown to be extremely social animals, and appear to be content in a group of compatible peers. Because most laboratories are not equipped to have animals multiply housed or even housed in pairs, an alternative is to give the animals human social contact through verbal and visual interaction (Sokol, 1993). Additionally, transport boxes and mirrors give nonhuman primates an opportunity to view, but not to contact, others of their species; hence, these devices can be used as environmental enhancement (Schapiro, Brent, Bloomsmith, & Satterfield, 1991).

An increase in adrenal cortisol has been correlated with suppression of the reproductive system, behavioral mobility, and environmentally directed activities (Moberg, 1985). Sustained activation of cortisol is believed to lead to increased susceptibility to infectious diseases, peptic ulcers, decreased reproductive capability, helplessness, and sudden death (Henry, 1976). The first researcher to develop a theory of the physiological component of stress was Hans Selye (1936), who formulated the concept of the General Adaptation Syndrome (GAS) which occurs after noxious stimuli are presented. The GAS is divided into three stages: an alarm reaction, a resistance stage, and exhaustion (Selye, 1936).

Stress has been objectively measured by analyzing plasma cortisol levels (Golub, Anderson, & Goo, 1981; Hanson, Larson, & Snowdon, 1976), but a preferable, non-invasive method of collecting cortisol samples is to measure urinary cortisol levels, which have been successfully used as an index of stress in nonhuman primates (Clark, Czekala, & Lindburg, 1995; Kling & Orbach, 1963; van Schaik, van Noordwijk, van Bragt, & Blankenstein, 1991).

Cortisol concentrations can be useful in evaluating the effects of an enrichment program for nonhuman primates, and researchers have sometimes evaluated psychological stress by measuring cortisol levels (Coe, Glass, Wiener, & Levine, 1983; Golub et al., 1981; Novak & Drewson, 1989; Schapiro,Bloomsmith, Kessel, & Keeling, 1991; Woolverton, Ator, Beardsley, & Carroll, 1989). Coe et al. (1983) suggested that cortisol levels be measured because hormonal responses may still be present long after the animal has habituated behaviorally to a stressful situation.

Serial Probe Recognition (SPR) has been used extensively to understand human cognitive processing (Roberts & Kraemer, 1981; Sands & Wright, 1980b; Waugh, 1960; Wickelgren & Norman, 1966), and is known to be sensitive to CNS damage in both human and nonhuman primates (Baddeley & Warrington, 1970; Gaffan & Weiskrantz, 1980). SPR has also been employed as a behavioral prototype for assessing cognitive function and to assess short-term memory in nonhuman primates (Castro & Finger, 1991; Finger & Kahler, 1992; Sands & Wright, 1980a; Sands and Wright, 1980b). SPR is a multiple-item memory test that measures sensory integration, vigilance, complex memory processing, and decision making ability (Castro & Finger, 1991; Waugh, 1960). In this institute, the SPR task was utilized to fulfill a requirement for a nonhuman primate behavioral task for an advanced screening of candidate compounds for the pretreatment and treatment against chemical warfare agents.

The purpose of the present study was to evaluate the effectiveness of the Serial Probe Recognition (SPR) task in providing environmental enrichment for nonhuman primates. Research has demonstrated that computerized tasks can be used effectively as environmental enrichment devices. In previous studies using the SPR task, the animals have displayed excitement at the onset of the task (e.g., by appearing impatient to enter the transport boxes and the SPR chambers). This reaction is considered to be a favorable response to the SPR task.

This study was expected to reveal that the urinary cortisol levels for the animals under the SPR condition would be less than the baseline measurements taken without SPR. The SPR task could then be considered an effective method of environmental enrichment.

Method

Participants

Twenty-one rhesus monkeys (Macaca mulatta) were assigned to two experimental groups: Group 1, males; no SPR training, followed by SPR learning (n=15); Group 2, females; no SPR training, followed by SPR learning (n=6). These animals were used as their own controls in this study. Data for the males and females were analyzed separately due to the cyclic nature of female hormones.

The rhesus monkeys were singly housed in stainless steel, squeeze-back cages (61 cm [W] X 71 cm [D] X 86 cm [H]). The cages were cleaned daily with a complete cage change every 2 weeks. The animal rooms were maintained at 20-22° C with a relative humidity of 50% (± 10%).

There were at least 10 complete air changes per hour of 100% conditioned fresh air. The animals rooms were on a 12:12-hr light/dark cycle with no twilight. All housing and care were in accordance with the "Guide for the Care and Use of Laboratory Animals" (U.S. Department of Health and Human Services, NIH, 1985). The animals were provided with a measureed ration (Certified Primate Chow Brand, Purina Mill, Inc., St. Louis, MO), water was available ad libitum, and their diet was supplemented with fresh fruit twice per day seven days per week.

Apparatus

The subjects were tested unrestrained in one of eight primate test chambers (61 cm [W] X 61 cm [H] X 61 cm [L]) constructed of Plexiglas and stainless steel. The walls of the test chambers were constructed of black Plexiglas and the top of clear Plexiglas, with the bottom made of stainless steel grid poles (1.5 cm diameter grid bars spaced 2.5 cm apart). The test boxes were housed in a sound and light attenuated chamber equipped with a ventilation fan. An Elographics (Oakridge, TN) serial touch screen (Model 002800A-131), 26.5 cm [W] X 21.5 cm [H], was attached to the front wall of each of the test boxes, adjacent to the entrance. A CXT high-resolution analog color video monitor (Model 1462ES) was positioned in the front wall of each of the experimental boxes so that the front of the monitor is encased by the touch screen. Reinforcement (300 mg banana flavored pellets, Bioserve Inc., Frenchtown, NJ) was delivered by a pellet dispenser (BRS/LVE Model QNB-4001) attached to the outside of the chamber and out of the animals' reach. The foodwell was positioned in the front of the test chambers, centered directly under the touch screen and 2 cm from the chamber floor. A speaker was located directly above the touch screen.

A Zenith 486 SX/25 microcomputer (Model ZMV-4492-KF) interfaced with the touch screen and was used to control all the experimental events and collect all the data. The stimuli consisted of 210 different images, such as animals, transportation vehicles, toy objects, food items, and other miscellaneous objects. The size of the objects ranged from 2.5 to 7 cm in length and were many different colors. Depending on the phase of the training, one or two stimulus objects were presented at the same time, one object above the other. The center for the top object was displayed 11.5 cm from the top of the touch screen and 7.5 cm from the left edge of the touch screen. The center for the bottom object was displayed 7.5 cm directly below the center of the top object. A white illuminated box (5 cm X 5 cm) was also displayed 24 cm from the top of the touch screen and 2 cm from the right edge of the touch screen. Throughout training and testing, all animals were monitored through the top of the primate test chambers using Panasonic cameras (Model WV-CP414).

Cortisol levels were measured by performing assays on the urine samples. A cortisol analyzer was purchased from Biochem Immuno-Systems, where the automated analyzer, the SR1, has been shown to be appropriate for the analysis of urine and serum (Bacarese-Hamilton, Cattini, Shandley, Howard, Palmer, & McFarthing, 1992). The SR1 spectrophtometrically quantitates the amount of cortisol in the sample. A wavelength absorbance is converted to a concentration based on a standard curve, and from that information, SR1 calculates the concentration of hormone or analyte in the solution (Biochem Immunosystems, 1994).

Procedure

An established enrichment plan continued throughout the entire study, which included the distribution of foraging devices, toys, and enrichment foods among all the animals in the study.

The 21 rhesus monkeys had their urine tested for a baseline measurement of cortisol for 10 working days prior to beginning the SPR task. The urine was collected twice per day for all animals, in the morning and in the afternoon. This was accomplished by placing a stainless steel pan (70 cm X 68 cm) under each of their enclosures. The urine was transferred to test tubes by means of pipettes and an extraction procedure was performed on the samples. The urine was thoroughly mixed and 500 ul was pipetted into a clean glass test tube to which 2.5 ml ice-cold dichloromethane (2 - 8° C) was added. The test tubes were then capped with glass stoppers. The samples were mixed gently on a vortex intermittently for 30 seconds (taking care to avoid the formation of emulsions), and the samples stood on ice for at least 2 minutes to allow the aqueous and organic phases to separate. Clean pipette tips were primed with ice-cold dichloromethane (2 - 8° C) and 2 X 750 ul was pipetted from the lower, organic layer into separate glass tubes. The solvent was then evaporated by passing nitrogen over the liquid. The residue was reconstituted with 300 ul of the SR1 Cortisol Calibration Kit Standard A (#163917-A) and mixed thoroughly to dissolve extract. The reconstituted extract was frozen at -84° C for approximately two weeks prior to assay. At the time of assay, samples were allowed to warm to room temperature (18 - 25° C) before being placed in SR1 cartridges. Samples that contain particulate matter were centrifuged at 1000 G for 5 minutes before being loaded into SR1 cartridges (Biochem Immunosystems, 1994).

The animals then began their SPR training, and approximately 2 weeks were allowed for the animals to acclimate to the procedure. During this time, all animals were taught to enter and exit an aluminum transport cage to permit transfer between their home enclosures and the test chambers. As soon as transfer training was complete, the animals were trained to press the touch screen with their hands, using standard shaping methods. Stimuli were presented on the touch screen along with a probe object, and the animal classified the objects (the stimulus and the probe) as either the same or different. If the objects were the same (a match), the animal was required to touch the screen area where the bottom object was displayed to receive a reinforcement. If the objects were different (non-matching), the animal was required to touch the white illuminated box to receive a reinforcement. Matching and non-matching trials were presented randomly and occurred with equal frequency throughout each session. When the animal responded correctly on 80% of the probe trials, new items were gradually introduced one at a time. This procedure would continue until six list items were presented.

When the animals were acclimated to the SPR training, based on the criteria that the animals entered and exited the transport cages and SPR chamber without hesitation or difficulty (approximately 2 weeks), urinary cortisol was measured in the morning prior to the SPR task and immediately after the SPR task, and these collection times were labelled 1st and 2nd.

Results

There were eight instances when it was impossible to collect a urine sample for an individual animal. The procedure of collecting samples required that urine be collected in the morning before SPR and immediately following SPR. Baseline samples were also collected at the approximate times that SPR samples were collected. If an animal had not given a urine sample within a reasonable amount of time (within 2 hours after collection pans being placed), its sample was given the value of zero for that collection time. It was necessary to fill in missing values because a repeated measures ANOVA could not be executed without a reduction in sample size. During the baseline condition, missing data were replaced with the mean of all data points for that particular time of day during that condition. For the SPR condition, missing data were replaced with the mean of the collection time immediately proceeding and after the missing point within that condition and particular time. It can be seen in Figures 1.a., 1.b., and 2.b. why this was appropriate (it is also statistically true of Figure 2.a.). The cortisol values during the baseline condition were relatively stable so that an overall mean was a reasonable estimate of the missing data point. During SPR, values varied widely, so it was necessary to determine individual means from previous plus following data as a way to estimate the missing data point.

Baseline values were then collapsed, and the overall 1st and 2nd means for the baseline condition were used to compare to the values of the SPR condition.

A sphericity test was applied to the data to determine whether the assumptions for the probabilities provided by the univariate F test were correct. Specifically, these tests require certain patterns of covariance matrices, known as Type H covariances. Data with these patterns in the covariance matrices satisfy the Huynh-Feldt condition. When the data satisfy the assumption of the Type H covariance matrices, the standard univariate F test is used to determine probability levels. If the sphericity test is significant, the data do not satisfy the Type H covariance assumptions, and an adjusted F test labeled the "Huynh-Feldt Epsilon" is used to determine probability levels (SAS Institute Inc., 1990). For the main effect of Day and the interaction of Day X Time, the data did not satisfy the sphericity test, and the adjusted "Huynh-Feldt Epsilon" was used as the F test. For the main effect of Time, the data did satisfy the sphericity test, and the standard univariate F test was used.

Analysis of the data used a 2 X 2 X 10 mixed factorial design with repeated measures on two factors (SPR and Baseline and the 10 sample collection trials).

Results showed that cortisol levels were significantly higher during the SPR condition than during the baseline condition, $F(10, 190) = 4.41$, $p = .001$. There was also a significant difference between 1st and 2nd measurements during the SPR condition, with 2nd cortisol levels being higher than 1st cortisol levels, $F(1,19) = 7.63$, $p = .0124$. In addition, there was a significant interaction of treatment condition and time of day during the SPR condition, $F(10, 190) = 3.53$, $p = .0079$, in that 2nd cortisol values during the SPR condition were significantly higher than the 2nd cortisol values during the baseline condition, and 1st cortisol values during the SPR condition were only slightly higher than 1st baseline cortisol values. The main effect for Sex was found not to be significant, and the interactions of Day X Sex, Time X Sex, and Day X Time X Sex were all found not to be significant (see Table 1).

Figure 1.a.

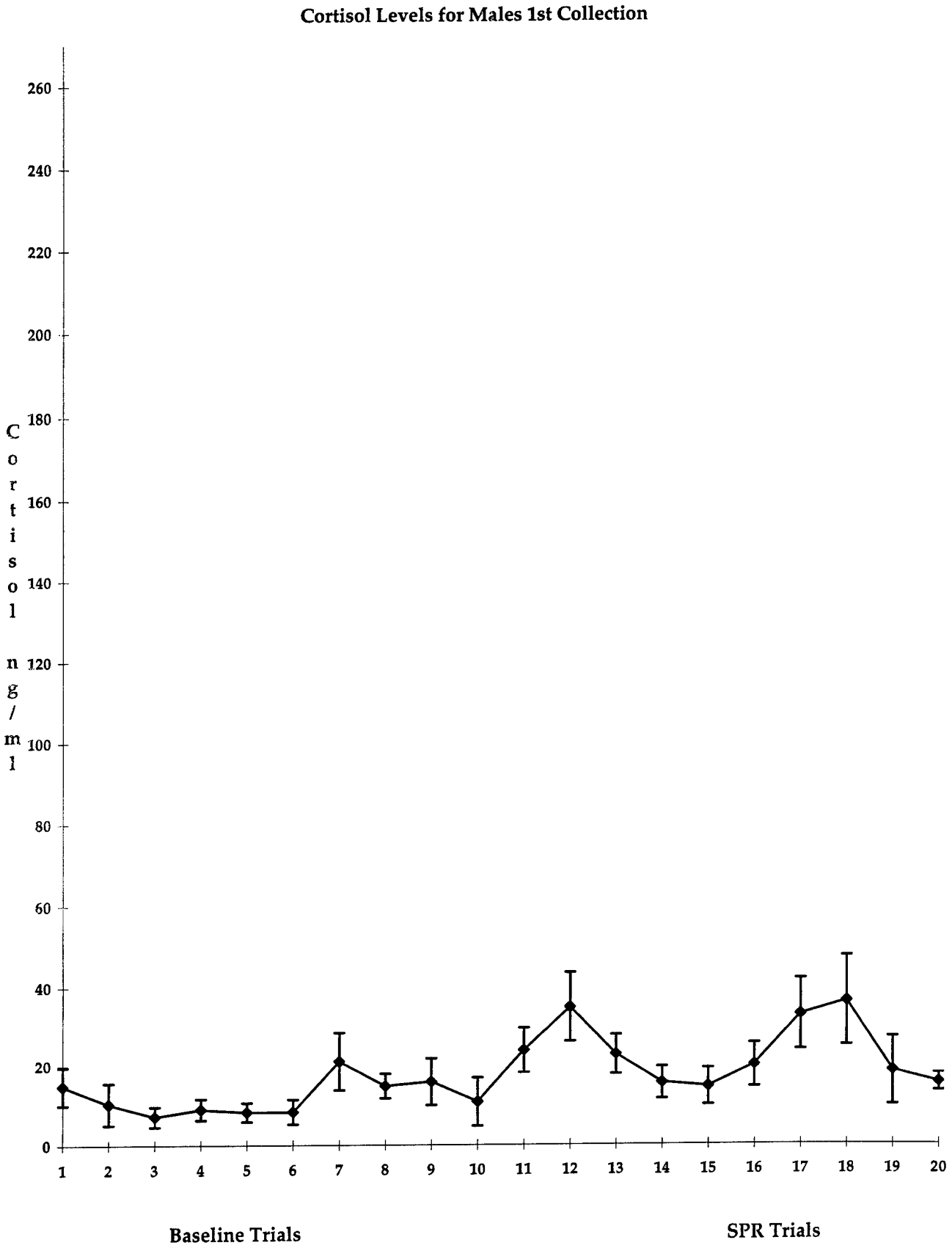

Figure 1.a. Trials 1-10 are baseline trials, and trials 11-20 are SPR trials.

Figure 1.b.

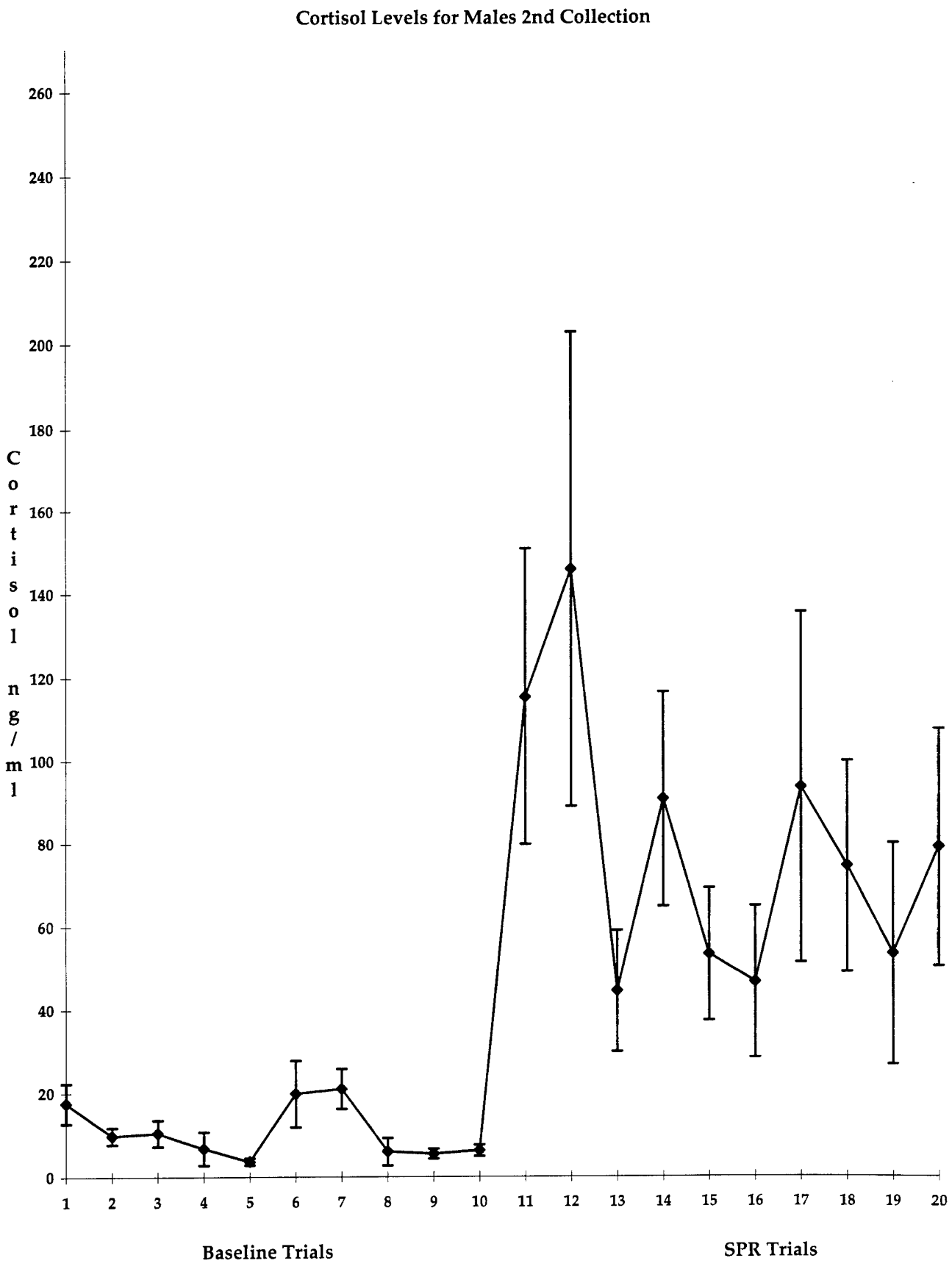

Figure 1.b. Trials 1-10 are baseline trials, and trials 11-20 are SPR trials.

Figure 2.a.

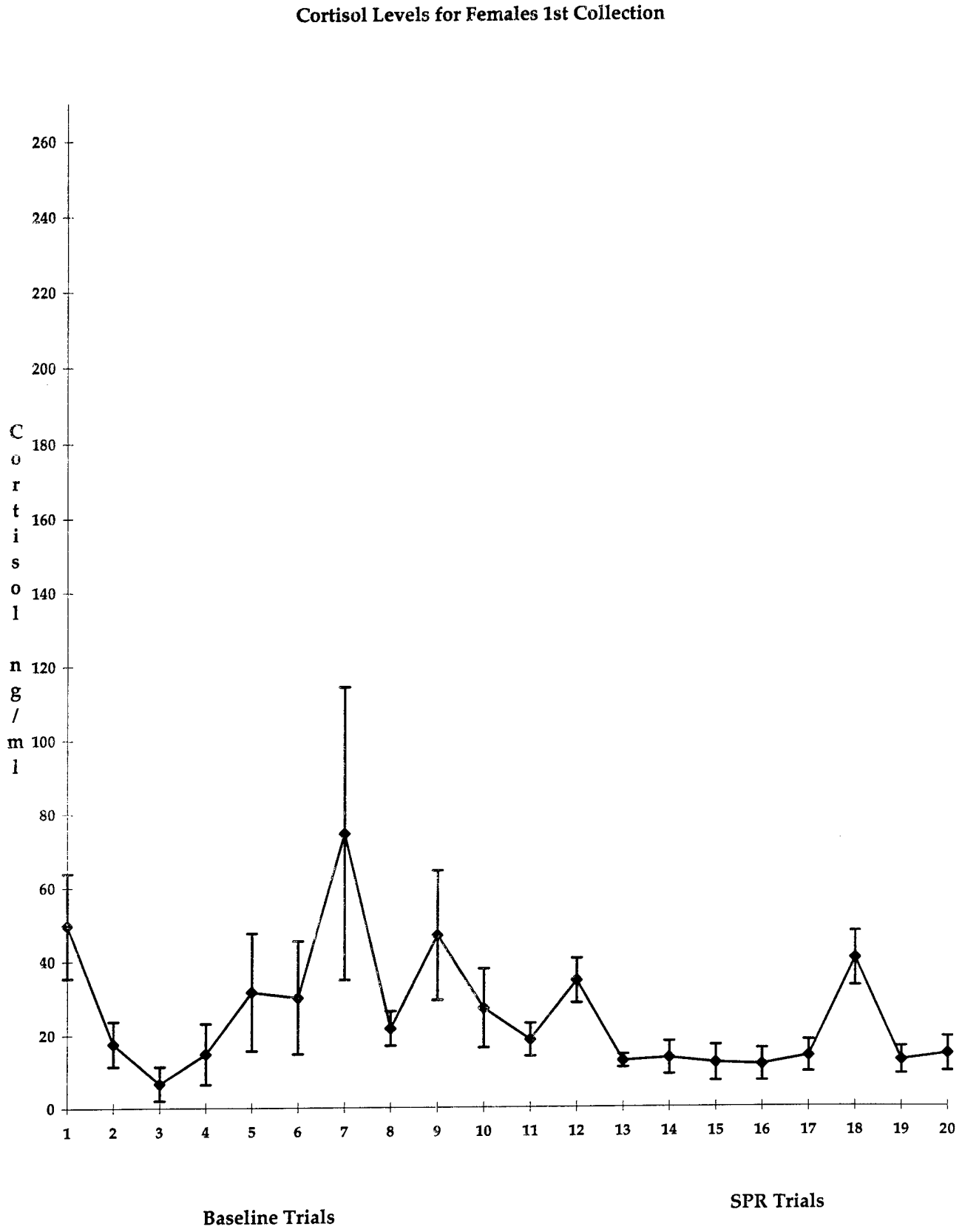

Figure 2.a. Trials 1-10 are baseline, and trials 11-20 are SPR trials.

Figure 2.b.

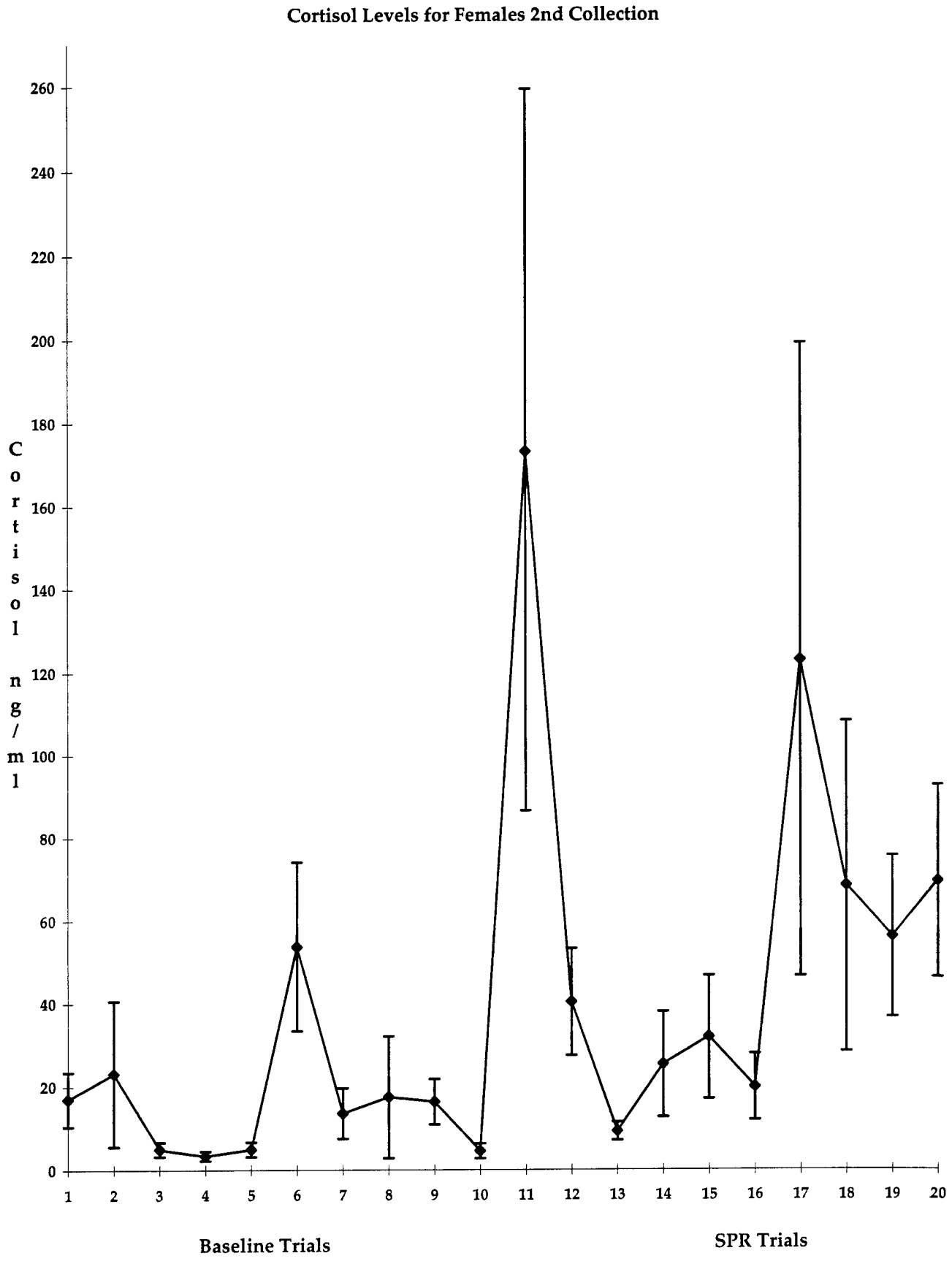

Figure 2.b. Trials 1-10 are baseline trials, and trials 11-20 are SPR trials.

A series of planned contrasts were conducted to compare the SPR condition to baseline. Due to the highly significant difference between them, 1st and 2nd cortisol values were analyzed separately (see Figure 3). During the 1st trials, days four, five, eight, and ten were significantly different from baseline. During the 2nd trials, days one, four, five, seven, eight, and ten were significantly different from baseline.

Discussion

The predicted outcome of urinary cortisol levels decreasing from baseline measured during SPR was not supported in the present study. Rather, there was a significant increase in urinary cortisol levels during the SPR condition, as well as a significant increase in urinary cortisol levels in the 2nd measurement as compared to 1st cortisol levels during the SPR condition. Several factors may have contributed to these results. First, the animals did not appear to have habituated physiologically to the SPR task when the experimental measurements were taken, and therefore, more time may have been needed between conditions. Second, the animals may not have been experiencing psychological distress during the SPR condition, but rather increased arousal as a result of the novelty introduced by the SPR task. Third, it is possible that the animals may have experienced some psychological stress as a result of the task. All three of these explanations would lead to increased cortisol levels.

When the present study was originally designed, it was determined that two weeks was an adequate waiting period to allow the animals to acclimate to the SPR task. This time period was believed reasonable to allow for training of the animals to learn how to use transport cages and to habituate to the SPR enclosures. After two weeks of acclimation, the animals did appear to habituate to the transport cages and to be acclimated to the SPR enclosures; however, urinary cortisol levels reflected a physiological state that was not behaviorally apparent. This finding is supported by Coe et al. (1983), who suggested that hormonal responses to stress may still be present long after the animal has habituated behaviorally to the stressful situation. During the SPR condition, the animals were continuing to be shaped to the SPR task according to conventional operant shaping procedures, which is to begin training with simple, easily executed components that are reinforced with food pellets to gradually develop the desired response. These changes may have contributed to the increased cortisol levels. Additionally, since individual animals were not completely shaped to the task, they may have experienced some frustration during the SPR task. This frustration may help explain why 2nd cortisol levels were significantly increased, as the animals may still have been aroused following the task due to the shaping procedures. Although 2nd urinary cortisol levels appear to reflect the effects of SPR procedures, 1st cortisol levels never decreased fully to baseline levels. This finding is consistent with the General Adaptation Syndrome (GAS), in that the animals' physiological state may be stabilized at the resistance stage, where corticoid levels remain at just above baseline (Selye, 1936).

It is worth exploring the possibility that the animals may not have been experiencing psychological distress, but rather increased physiological arousal necessary for adaption to their environment. A certain amount of stress appears to be necessary for performance of a task, and more over, arousal theory states that an optimal level of arousal exists at which behavior will be

Table 1.

ANOVA TABLES

Source: Day

DF	Type III SS	Means Square	F Value	Adj. Pr > F H - F
10	151212.482	15121.2482444	4.41	0.0010

Source: Time

DF	Type III SS	Means Square	F Value	Pr > F
1	185006.547	185006.547	7.63	0.0124

Source: Sex

DF	Type III SS	Means Square	F Value	Pr > F
1	8340.13903	8340.13903	0.22	0.6452

Source: Day X Time

DF	Type III SS	Means Square	F Value	Adj. Pr > F H - F
10	114585.767	11458.5766783	3.53	0.0079

Source: Day X Sex

DF	Type III SS	Means Square	F Value	Adj. Pr > F H - F
10	41534.757	4153.4757820	1.21	0.3097

Source: Time X Sex

DF	Type III SS	Means Square	F Value	Pr > F
1	4027.651	4027.651	0.17	0.6881

Source: Day X Time X Sex

DF	Type III SS	Means Square	F Value	Adj. Pr > F Pr > F
10	45598.823	4559.882	1.41	0.2347

Figure 3.

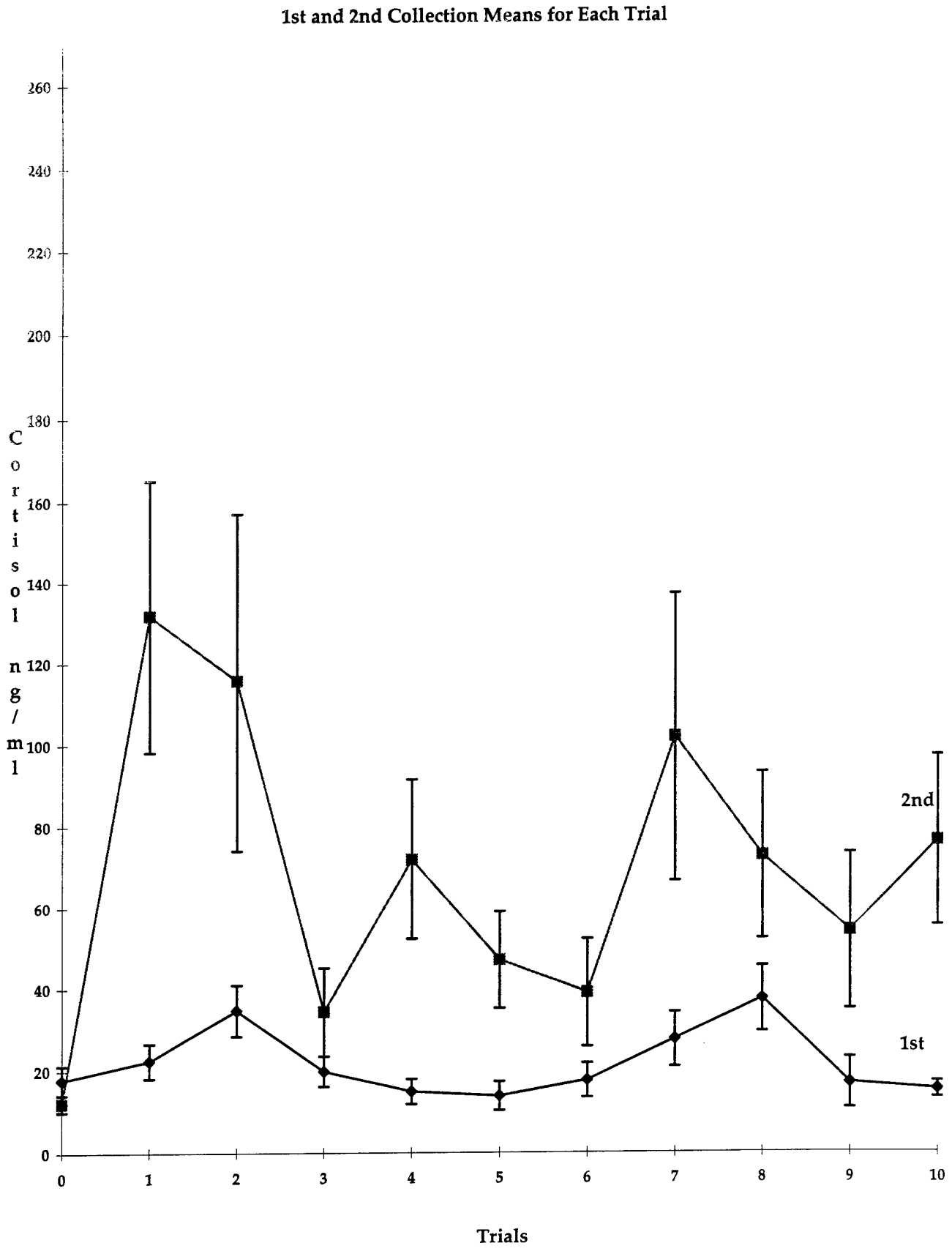

Figure 3. Trial 0 are the collapsed 1st and 2nd baseline values, and trials 1-10 are the 1st and 2nd SPR values.

most efficient. This theory is often called the Yerkes-Dodson Law, and it is depicted as an inverted U function. This "law" indicates that increased arousal improves performance only up to a point, after which continued increases in arousal tend to impair functioning (Hebb, 1955). The increased urinary cortisol levels in the present experiment may have been the result of the increased demands of a novel task. This increased arousal might also explain the increased cortisol levels in the 2nd measurement.

A final possibility for the increased cortisol levels during the SPR condition may be that the animals were in psychological distress, and that SPR is a noxious stimulus. The GAS may also explain this phenomenon. During the alarm reaction, organisms respond to noxious stimuli by exhibiting changes in the adrenal gland, gastrointestinal tract, and lymph system. These changes accelerate breathing and heart rate and increase blood pressure. The resistance stage then occurs, and corticoid levels drop to just above baseline. Upon discussing performance of the animals with the SPR technicians at approximately six weeks following the completion of the present study, it was reported that the animals are now considerably calmer, more solicitous of the technicians in positive ways (i.e., presenting their rumps for scratching and reaching out their hands in a gentle manner), less vocal, and less excitable. It is possible that the animals are now fully habituated to the SPR task, and it is also possible that they have habituated to the task physiologically as well. These observations do not suggest that SPR is currently a noxious stimulus or that it ever was.

Given the information presented in this study, the author concludes that the best models to explain why the animals experienced an increase in cortisol levels are 1) the assertions that the animals had not habituated to the SPR task when experimental measurements were taken and 2) the animals may have been experiencing the increased arousal necessary to perform a novel task. The first model is supported by the fact that the animals were not successfully shaped to the task. As stated previously, these procedures appear to have been contributing to the increased stress level of the animals, and consequently, increased cortisol levels. The second model is supported by theYerkes-Dodson Law (Hebb, 1955), which states that a certain level of arousal is necessary to perform a novel task, and due to the relative inactivity of the animals before the onset of this study, this model presents a strong argument to explain the increased cortisol levels. Therefore, the results of the present study suggest that future research include examining cortisol levels at regular intervals throughout imminent SPR projects. This may give more information about the animals' responses to SPR, and determine more effectively whether SPR can be thought of as an environmental enrichment device. Additionally, looking at cortisol levels throughout SPR projects may give information regarding the stress levels of the animals and their ability to adequately perform the SPR task.

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